Polyvinyl Alcohol Nephrosis: Relationship of Degree of Polymerization to Pathophysiologic Effects.* (27958)

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Subcutaneous administration of aqueous solutions of polyvinyl alcohol having a molecular weight of 133,000 to rats maintained on a high NaCl diet produces a nephrotic syndrome, characterized by hypertension, edema, ascites, proteinuria, and anemia(1-3). Grossly the kidneys are usually pale, yellow and granular and often show small punctate surface hemorrhages. Microscopically, cells of the glomerular endothelium and epithelium are swollen to form "foam cells"(2-4). There is endothelial and epithelial proliferation, formation of glomerular crescents, hyalinization of glomeruli, glomerulosclerosis and glomerular hemorrhages, often associated with interstitial nephritis or even pyelonephritis(2,3). The polysaccharide lodges within glomeruli, tubular lumens and interstitial spaces. Cardiac hypertrophy is accompanied by sclerosis and hyalinization of cardiac arteries, and similar vascular lesions are produced in the spleen, pancreas, mesentery and brain(2,3). The polysaccharide infiltrates the myocardium and also the spleen and liver where it produces hepatosplenomegaly(2,3).

To determine whether the degree of polymerization had an important bearing on the induced changes it was decided to employ larger and smaller molecular weight polymers from the same manufacturer, and compare the response obtained to that resulting from administration of the material of known efficacy.

Materials and methods. Forty-six young female rats of the Holtzman strain were divided into 4 groups. Groups 1, 2 and 3 each received a daily subcutaneous injection of 1 ml of 5% polyvinyl alcohol (PVA) dissolved in physiological saline, whereas those of Group 4 received the same amount of vehicle. The PVA† given to Group 1 was grade 51-05, Group 2 was given grade 72-60 and Group 3 received grade 71-30. These are respectively described by the manufacturer as being polymers of low molecular weight (37,000) low viscosity (4-6 centipoises); high molecular weight (185,000) high viscosity (55-65 centipoises); and medium molecular weight (133,000) medium viscosity (28-32 centipoises).

The animals were given a diet of Purina Laboratory Chow *ad libitum* and a 1% solution of NaCl in distilled water to drink. They were individually caged in temperature controlled quarters. Fluid intakes were measured daily. Blood pressures of unanesthetized animals were measured weekly with a tail plethysmograph, and systolic pressures in excess of 150 mm Hg were regarded as hypertensive.

Animals were killed with ether on the 29th day and various tissues and organs were taken for weight and/or histology. Tissues and organs were fixed in 10% neutral formalin and, with the exception of the liver which was weighed fresh, were removed after fixation, blotted dry, dissected free and weighed on an analytical balance. Histologic sections were stained with Congo red-hematoxylin and with hematoxylin and phloxin.

Results. All rats given the medium grade of PVA developed a prompt polydipsia, and a progressively severe hypertension. The ultimate pressures achieved were 200 mm of Hg or greater. The 2 other polymers had a lesser effect upon blood pressure and little if any effect upon fluid intake. Four animals given the smallest polymer and 6 treated with the largest polymer had terminal pressures between 150 and 166 mm of Hg. The rest of both groups and all of the controls remained normotensive. Fluid intakes are given in Fig. 1 and blood pressures in Fig. 2.

All 3 PVA solutions, but particularly the large and medium molecular weight polymers,

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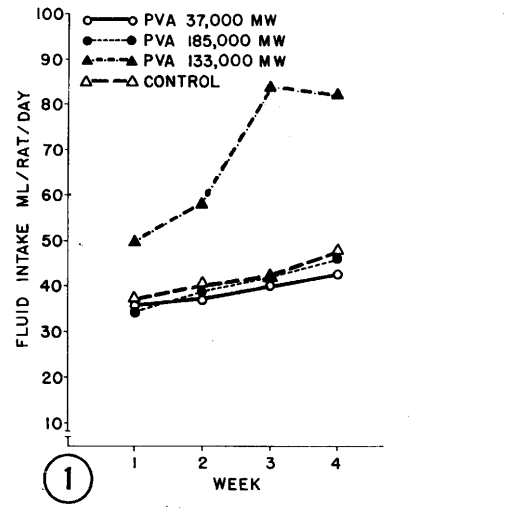
† Elvanol, I. E. du Pont de Nemours, Inc.



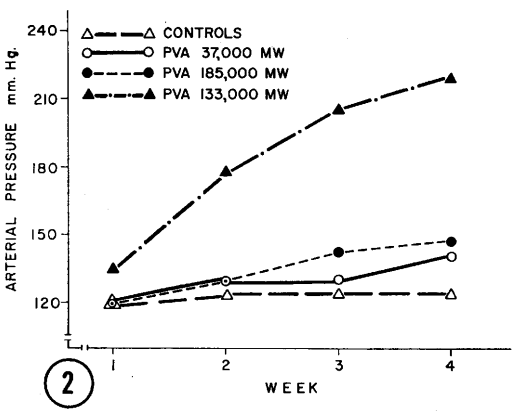


FIG. 1. Weekly average of daily fluid intake of control and PVA-treated rats. Note induction of polydipsia by 133,000 MW polymer and lack of effect by larger or smaller molecules.

FIG. 2. Blood pressure of control and PVA-treated rats. Note that response to large and small polymers is slight and equivalent. Medium molecular weight PVA causes rapid onset and progression of hypertension.

caused extensive but temporary loss of hair in the area of subcutaneous injection. This reached a maximum severity in from 5 to 12 days after injections began, at which time areas of 2-6 sq cm were almost entirely denuded. Recovery was equally dramatic despite continued injections, and by the end of the experiment the pelage was normally luxuriant. During the experiment marked abdominal swelling, suggesting ascites, was noted in most of the animals receiving the medium weight polymer, and the subcutaneous tissues were often thick and edematous. These animals lost weight terminally.

At necropsy a marked accumulation of ascitic fluid, often associated with thoracic fluid and peripheral edema, was noted within the abdominal cavity of several animals given the medium molecular weight material. The kidneys were pale, enlarged and granular, and often bore pin-point hemorrhages on the surface. Marked edema of the pancreas, mesentery and intestinal tract affected several of them. Epicardial scars were visible on the surface of the enlarged cardiac ventricles. Hepatosplenomegaly was characteristically present. A less marked hepatosplenomegaly and renal enlargement was also noted in all animals given the largest polymer. Otherwise the tissues of this group were not grossly abnormal. There were no noteworthy changes among controls or in animals which had been given the smallest of the polymers.

Organ weights revealed that only the medium molecular weight material caused significant cardiac ($P<.001$) hypertrophy. It also produced the most marked hepatic ($P<.001$), renal ($P<.001$) and splenic ($P<.001$) enlargement and the greatest atrophy of the thymus. The adrenals were not significantly ($P>.25$) enlarged. The high molecular weight PVA also caused significant enlargement of the heart ($P<.02$), liver ($P<.001$), kidneys ($P<.001$) and spleen ($P<.01$), whereas the small molecular weight did not. The data are summarized in Table I.

Histologic examination of tissues with the Congo-red stain, which has marked affinity for PVA(2) revealed no trace of the small molecular weight material in sections of the adrenal, kidney, heart, spleen or liver. Nor were there pathologic abnormalities in any of these organs. The intermediate polymer produced severe renal, cardiac and splenic arterial lesions in all animals. These have been described elsewhere and imputed to the effects of hypertension(2,3). Infiltration of the material into the adrenal medulla, spleen, myocardium, liver and kidney (Fig. 3) was noted in confirmation of earlier findings(2,3). Glomerular enlargement and hyalinization,

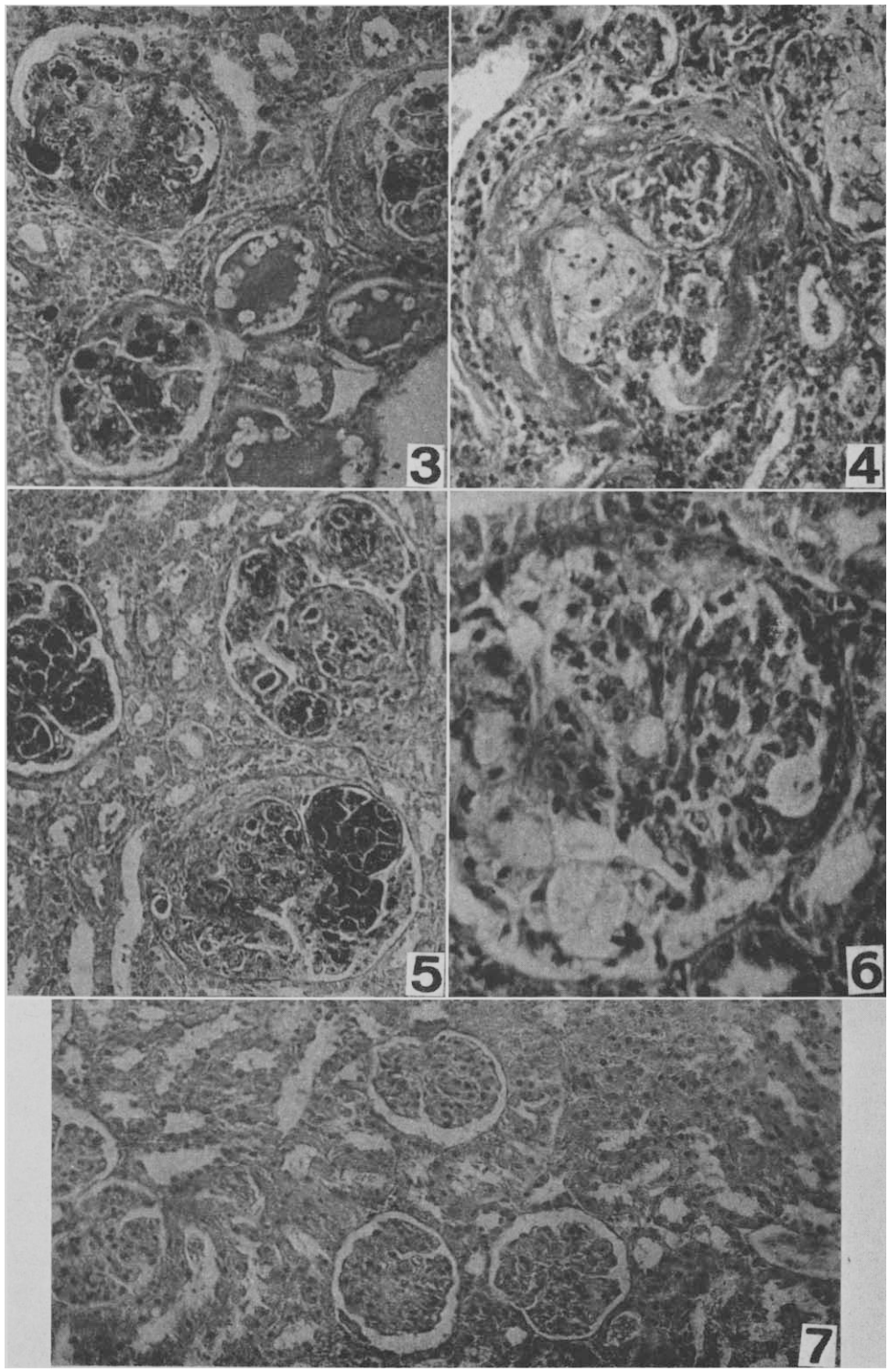


FIG. 3. Kidney section from rat given 133,000 MW PVA. Glomeruli are enlarged and contain large amounts of the polysaccharide. Hyalinized crescent is present in glomerulus at

right. Tubules are dilated and contain hyaline casts. Congo red-hematoxylin × 160.

FIG. 4. Severely damaged glomerulus from rat treated as in Fig. 3. Collagenized epithelial cells appear at upper right margin of Bowman's capsule. Glomerulus contains much fibrinoid material and is hemorrhagic. Hematoxylin and phloxin × 198.

FIG. 5. Kidney section from rat given 185,000 MW PVA. Glomeruli enlarged, infiltrated and adherent to capsule as in Fig. 3, but note that tubules are essentially normal. Congo red-hematoxylin × 160.

FIG. 6. Glomerulus from same rat as in Fig. 5. Note foam cells and hyaline transformation of capillary walls and wall of Bowman's capsule. Hematoxylin and phloxin × 400.

FIG. 7. Kidney section from rat given 37,000 MW PVA. Glomeruli and tubules have a normal appearance. Congo red-hematoxylin × 160.

capillary sclerosis, crescent formation and hemorrhage were characteristic (Fig. 4). Hyaline casts were common in tubular lumens which sometimes contained polymorphonuclear leucocytes. Interstitial nephritis was noted in several instances.

The largest polymer also accumulated in the same organs, but the extent of the encroachment, with the exception of the renal glomeruli which appeared to be equally swollen and infiltrated (Fig. 5), was substantially less marked. Glomerular hyalinization or hyaline casts were rarely seen in the kidneys of normotensive animals, but were not uncommon in those from hypertensives (Fig. 6). Proliferation of the capsular epithelium and adhesion of glomerular tufts to the capsular wall were frequent among hypertensives, but neither interstitial nephritis nor glomerular hemorrhage were observed to occur in any animal. Although the renal glomeruli appeared to have substantially identical amounts of polyvinyl alcohol deposits within them as was the case with the medium molecular weight material, they appeared to be much less damaged. Tubular casts were of infrequent occurrence and both tubular dilation and interstitial PVA accumulation were less marked. The large molecular weight PVA caused no extrarenal vascular lesions, nor apart from the mere presence of the polysaccharide, were lesions observed in any other organs of treated animals. The kidneys of rats given the small molecular weight PVA were normal in appearance (Fig. 7).

Discussion. The first experiment, in which PVA with a molecular weight of 133,000 was employed, revealed that hypertension was associated with renal damage(1,2). It was concluded that the elevation of blood pressure here, as in the case of methylcellulose administration which causes a similar hypertensive-nephrotic syndrome and comparable kidney changes(5-7), might well be caused by a renal mechanism evoked by the glomerular epithelial and endothelial swelling(1,2). The force of this hypothesis as the sole explanation is weakened by the present findings. Hypertension, admittedly of mild degree, occurred in 33% of the rats treated with the lowest molecular weight PVA even though it neither invaded nor caused a detectable change in any organ. Furthermore the heaviest PVA, although it infiltrated the same organs as did the medium molecular weight material and caused comparable glomerular

TABLE I. Principal Effects of Subcutaneously Administered Polyvinyl Alcohol.

		Molecular wt			
		37,000	185,000	133,000	Controls
Body wt, g	Initial	82 ± 2*	82 ± 2	84 ± 2	82 ± 1
	Final	180 ± 5	182 ± 3	164 ± 7	190 ± 4
No. rats	Initial	12	12	10	12
	Final	12	12	10	12
Organ wt, g/100 g body wt	Liver	5.93 ± .08	6.36 ± .16	7.15 ± .20	5.51 ± .08
	Kidneys	1.166 ± .03	1.466 ± .07	2.598 ± .08	1.166 ± .02
mg/100 g body wt	Spleen	323 ±10	352 ±19	632 ± 69	301 ± 7
	Heart	353 ± 4	366 ± 9	534 ± 13	340 ± 5
	Thymus	216 ± 7	189 ± 9	170 ± 22	240 ±11
	Adrenals	37.1± 1.4	35.5± 1.1	39.4± 2.9	37.2± .8

* Mean ± S.E. of mean.

effects, was only slightly more effective than the smallest polymer in causing hypertension even though it did cause sclerosis and hyalinization of glomerular capillaries.

Greatest renal and vascular damage was induced by the intermediate PVA, probably because the effects of incorporation and hypertension were additive. Consequently arteriosclerosis, cardiac lesions, interstitial nephritis, glomerular lesions and hemorrhage and marked enlargement of the heart, kidney, liver and spleen were characteristic findings. Incorporation of high molecular weight PVA by spleen, liver, heart and kidneys led to enlargement of these organs, but not to the degree found with the intermediate material. These organs were not affected by the smallest molecular weight PVA, and they apparently contained none of it.

The intermediate polymer was the only one which produced polydipsia, hence animals thus treated consumed, on the average, far more NaCl than did those of other groups. Within the groups there was no definite correlation between amount of fluid consumed and blood pressure. In Group 3 there were three animals which consistently consumed about half as much as any of the others, yet had as severe hypertension. This polymer also causes severe hypertension in animals given distilled water to drink(5). For these reasons it seems unlikely that hypertension in PVA treated rats can be ascribed merely to an increased sensitivity to the hypertensive effects of NaCl(8,9) although it is evident that substitution of 1% NaCl for distilled water as drinking fluid enhances the hypertensive and pathologic effects of PVA treatment(5).

Conceivably the adrenal cortex may participate in the genesis of hypertension by PVA, although its slight enlargement in hypertensive animals, especially evident with the 133,000 MW material here as in other studies, can as justifiably be ascribed to the results of hypertensive disease as to the cause.

Polyvinyl alcohol is known to form viscous solutions which coat the intima of blood vessels and erythrocytes. It may well be that a part of its hypertensive effect is due to the viscosity effects or to an induced osmotic hydremia(10).

Evidently the hypertensive and other pathologic effects of PVA are strongly conditioned by degree of polymerization, and hence physical properties, and are less dependent upon chemical structure. The 37,000 MW material may be efficiently filtered by the kidney and excreted. The intermediate and high molecular weight polysaccharides are apparently too large to be effectively filtered, and hence lodge within the glomeruli causing glomerular damage, the former causing a greater renal injury. This is accompanied by the changes of glomerulonephritis which, when severe, allow the material to escape into the tubular lumens and form casts. Retained PVA also infiltrates other organs where it is taken up by elements of the reticuloendothelial system. Although both the medium and large polymers widely infiltrated various organs and tissues and both caused renal damage, only the former resulted in development of a nephrotic syndrome accompanied by severe hypertension.

Summary. Polyvinyl alcohol of 3 different molecular weights, 37,000, 133,000 and 185,000, was injected daily and subcutaneously into rats on a high NaCl intake. The lowest molecular weight material was not demonstrated in any of the tissues examined and, over the period studied, produced only mild elevation of blood pressure in a third of the animals which received it. High molecular weight PVA infiltrated a number of organs and tissues. Most prominently it affected renal glomeruli causing swelling and multiplication of endothelial and epithelial cells and often crescent formation. Mild hypertension affected half of the animals and heart, kidneys, liver and spleen were enlarged. Neither polymer caused polydipsia. The intermediate polymer produced severe polydipsia, a nephrotic syndrome associated with ascites and edema, severe hypertension, marked renal damage with severe glomerulonephritis, often hemorrhagic, and widespread cardiovascular lesions. The pathologic effects depend more upon the molecular size than upon chemical structure.

1. Hall, C. E., Hall, O., *Experientia,* 1962, v18, 38.
2. ———, *Am. J. Path.,* 1962, v41, 247.
3. ———, *Lab. Invest.,* 1962, v11, 826.
4. Hueper, W. C., *Arch. Path.,* 1939, v28, 510.
5. Hall, C. E., Hall, O., *Am. J. Path.,* 1962, v40, 167.
6. ———, *Texas Rep. Biol. & Med.,* 1962, v20, 185.
7. ———, *ibid.,* 1962, v20, 587.
8. Meneely, G. R., Tucker, R. G., Darby, W. J., Ruerbach, S., *J. Exp. Med.,* 1953, v98, 71.
9. Koletsky, S., *Lab. Invest.,* 1958, v7, 377.
10. Hueper, W. C., *Arch. Path.,* 1942, v33, 267.

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Adjuvant Arthritis Induced in Germ-Free Rats.* (27959)

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A polyarthritis induced in rats by a single injection of Freund's water-in-oil adjuvant has been under investigation since 1956(1) but the precise mechanisms which underlie its development are still obscure. It is most likely that delayed hypersensitivity is implicated but the antigenic stimulus, if any is involved, has not been found. In the background in all of the experiments lingers the possibility that a latent infectious agent has been aroused by the injected adjuvant (which is itself sterile), thereby causing an infectious arthritis. Because of this possibility considerable effort has been expended by us (2) and others(3) in an attempt to clarify this matter. These studies have not shown bacteria, fungi or pleuropneumonia organisms (PPLO or Mycoplasma) when a wide variety of cultural technics were used on joint fluid, periarticular tissues or visceral organs. The studies did not rule out the presence of viruses, rickettsiae or certain bacteria or other microbes with fastidious growth requirements, but the presence of the latter seemed unlikely.

To pursue this matter further adjuvant was given to a group of "germ-free" animals so that the effect of the normal bacterial flora, or lack thereof, could be assessed in relation to the induced arthritis. Germ-free animals differ physiologically from "conventional" animals maintained in a non-sterile environment in several respects including the presence of lower concentrations of circulating beta and gamma globulins(4) and some retardation in their immune responses(5).

Five male and four female rats of the Lobund strain, which have been maintained in the germ-free state for 12 generations, and housed at the NIH, were used in the study. At time of inoculation their weights ranged from 131 to 166 g. Other details of germ-free existence and measures necessary to maintain the animals in a healthy state have been outlined elsewhere(6). The adjuvant preparation has also been described previously(7). In brief it contained 1 mg of the Wax D fraction† of human *M. tuberculosis* (strain Brevannes) per ml of final mixture; 0.5 ml was given subcutaneously into the proximal half of the tail in a single injection.

Except for slight nodular swelling which developed along the site of adjuvant deposition on about the 8th day and necrosis of the distal 1 or 2 cm of the tail which occurred in 6 of the animals on about the 6th day the animals appeared well until the 16th day when 2 showed minimal arthritic reaction in one or more paws or digits. On the 18th day another 2 animals had arthritis and on the 20th day 9/9 were affected. In all ani-

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